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Effects of Alprazolam, Zolpidem and Zopiclone, and of chronic inflammation on peripheral experimental algesia in Wistar rats

MIHAELA ZDRÎNCĂ[1)], GABRIELA MUŢIU[2)], MARIA BOGDAN[3)], LUCIANA DOBJANSCHI[1)], ANGELA ANTONESCU[4)], IOANA MOŞ[5)], MARIANA MUREŞAN[4)], M. ZDRÎNCĂ[6)], ANDREEA ANTONESCU[7)]

[1)]*Department of Pharmacology*
[2)]*Department of Histology*
Faculty of Medicine and Pharmacy, University of Oradea
[3)]*Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova*
[4)]*Department of Biochemistry*
[5)]*Department of Microbiology*
[6)]*Department of Surgery*
[7)]*Student*
Faculty of Medicine and Pharmacy, University of Oradea

Abstract

In the literature, there are some data which indicate that benzodiazepines and other chemical compounds with the same mechanism of action (Diazepam, Chlordiazepoxide, Lorazepam, Zopiclone, etc.) also have other effects. We investigated the effects of experimental chronic inflammation under the administration of some tranquilizers and hypnotics on peripheral algesia induced in rats by "writhing test". Chronic inflammation was induced by "cotton wool granuloma" technique. The "writhing test" consisted in intraperitoneal injection of an irritant agent (acetic acid 0.0025%, 0.4 mL). The animal reacts with a characteristic stretching behavior called writhing. A writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Then, the animals were placed individually into glass beakers and 5 minutes were allowed to elapse. The rats were then observed for a period of 10 minutes and the number of writhes is recorded for each animal. Three drugs were administered by gastric probe: Alprazolam 1 mg/kg, Zolpidem 10 mg/kg and Zopiclone 10 mg/kg. Alprazolam is a triazolobenzodiazepine derivative used as a tranquilizer. Zolpidem is an imidazopyridine with marked sedative-hypnotic effect and it has the same mechanism of action like benzodiazepines. Zopiclone is a cyclopyrrolone with sedative-hypnotic effect used as hypnotic and acts like benzodiazepines. After that, the animals were sacrificed and the weight of cotton wool granuloma was determined. In the same time, the histopatological aspect of granulomatous inflammation was studied. It was found that experimental proliferative inflammation under the action of these drugs was accompanied by a peripheral analgesic activity in "writhing test". The mechanisms of these effects are not fully elucidated. Some explanations are: they act as agonists or antagonists on algesia and inflammation mediators and they have a stimulating effect on peripheral ω_3-benzodiazepine receptors ("peripheral-type" receptors).

Keywords: Alprazolam, Zolpidem, Zopiclone, rats, chronic inflammation, algesia.

Introduction

The medical literature mentions very few data about the analgesic or algesic effects of benzodiazepines or other drugs with similar mechanism of action.

Alprazolam is a triazolobenzodiazepine derivative used as a tranquilizer. It acts by binding to benzodiazepine binding sites on $GABA_A$-receptors and stimulates the inhibitory action of gamma-aminobutyric acid (GABA) in CNS [1–7]. Tranquilizers are used in the treatment of anxiety (in various types of neurosis, depressions), in management of seizure disorders, treatment of alcohol withdrawal syndromes, as central skeletal muscle relaxants, as anesthetic premedication. Alprazolam is also used in panic attacks [3, 5–9].

Zolpidem is an imidazopyridine with marked sedative-hypnotic effect and acts like benzodiazepines (BZD) by binding to BZD receptors and stimulating the inhibitory activity of GABA in the central nervous system [1–3, 5, 6].

Zopiclone is a cyclopyrrolone with sedative-hypnotic, anxiolytic, anticonvulsant and myorelaxant properties. It is used as a hypnotic and acts like BZD (by stimulation of the central BZD receptors) [1–3, 5, 7]. In the literature, there are some data which indicate that benzodiazepines (Diazepam, Chlordiazepoxide, Lorazepam, etc.) also have other effects: they have the property to lower the elevated serum lipids (the serum total lipids, the total cholesterol and especially the triglycerides) when they were raised by various methods [10, 11]; they reduce hyperglycemia induced by intravenous administration of streptozotocin (due to stimulation of glucose uptake into tissues); some BZD stimulate

fibrinolysis, decrease blood viscosity and have anti-aggregant action. Previous investigations have shown that Zopiclone also induces a very significant reduction of total lipids, total cholesterol and triglycerides in conditions of hyperlipidemia [10, 11].

Certain doses cause a decrease of blood glucose level when it was increased. These effects are explained probably by stimulation of peripheral benzodiazepine receptors.

Zolpidem was also investigated in hyperlipidemia induced by Triton WR–1339 administration. The results were approximately the same as those induced by Zopiclone.

In this paper, we present the results of the investigations of the effects of experimental chronic inflammation under the administration of these three drugs (Alprazolam, Zopiclone and Zolpidem) on peripheral algesia induced in rats by the writhing test.

Materials and Methods

The experiments were carried out on Wistar rats of both genders. The animals were divided into four groups of six weighing 150–190 g (Table 1) and were kept in normal laboratory conditions.

Table 1 – ***Study groups***

Group no.	Characteristics	No. of animals
1.	*Control*	6
2.	*Alprazolam*	6
3.	*Zopiclone*	6
4.	*Zolpidem*	6

Group 1 (six animals) was the control group and they were kept in normal laboratory conditions.

For a 3-day period, the animals in the other three groups were administered different medications, as follows:

- Alprazolam (1 mg/kg) was administered to the second group (six animals) by gastric probe;
- Zolpidem (10 mg/kg) was administered to the third group (six animals) by gastric probe;
- Zopiclone (10 mg/kg) was administered to the fourth group (six animals), also by a gastric probe.

In the first day of the experiment, the animals were also superficially anesthetized with ether until muscle relaxation was observed.

Then, an experimental chronic inflammation was produced using the "cotton wool granuloma" method. After that, for 3 days, the animals were kept in normal laboratory conditions.

In the 4th day, the "writhing test" [12] was applied to each rat in these four groups consisting in: 0.4 mL of 0.025% acetic acid solution injected intraperitoneally in each animal and the time of administration was noted. Then, each animal was placed in a glass bowl.

After 5 minutes from the administration, the time of the onset of abdominal contractions and the number of contractions for a period of 10 minutes were noted.

After that, the animals were sacrificed and the weight of the cotton wool granuloma was determined. In the same time, the presence of the foreign body inflammatory reaction studied.

The comparison was made between the control group (with chronic inflammation and writhing test) and the groups treated with drugs (with chronic inflammation and writhing test).

We compared the onset of abdominal contractions in the control group and the groups treated with drugs, the frequency of abdominal contractions in the control group and the groups treated with drugs, and the weight of the cotton wool granuloma in the control group and the groups treated with drugs.

The statistical analysis was performed using the Kolmogorov–Smirnov test, the Student *t*-test and the ANOVA method [3, 5].

Results

The time of the onset of abdominal contractions in all groups (control group and groups treated with Alprazolam, Zolpidem and Zopiclone) and the mean time of muscle contraction onset was calculated (Table 2 and Figure 1) [9].

Table 2 – ***Mean time of on traction onset***

Group	Mean time [min] ± SD
Control	2.50 ± 1.51
Zopiclone	3.50 ± 1.38
Zolpidem	1.83 ± 0.75
Alprazolam	3.25 ± 1.17

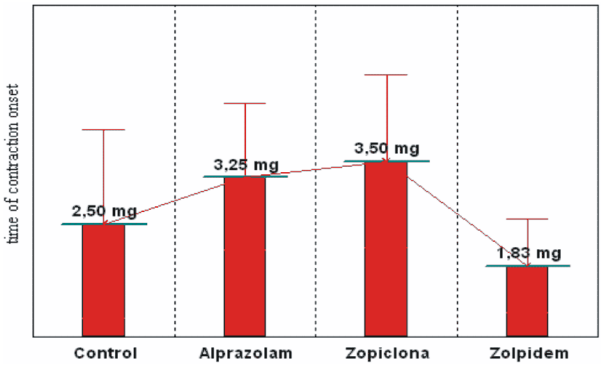

Figure 1 – ***Mean time of contraction onset.***

We compared the control group (with chronic inflammation and writhing test) and the groups treated with drugs (with chronic inflammation and writhing test) (Table 3).

Table 3 – ***Comparison between mean times of contraction onset***

	Control	Alprazolam	Zopiclone	Zolpidem
Control		0.778	0.591	0.832
Alprazolam			0.989	0.300
Zopiclone				0.178

There were no significant differences between the control group and the groups treated with Alprazolam, Zolpidem and Zopiclone and between the groups treated with different drugs.

Then, we noted the number of abdominal muscle contractions for a period of 10 minutes in each animal from each group and the mean frequency of abdominal contraction was determined (Table 4 and Figure 2) [9].

We compared the mean frequencies in the control group and the groups treated with drugs (Table 5) [9].

Table 4 – *The mean frequency of abdominal contractions*

Group	Mean number of contractions/10 min. ± SD
Control	470.50 ± 59.47
Zopiclone	24.67 ± 2.31
Zolpidem	137.83 ± 15.83
Alprazolam	77.83 ± 8.36

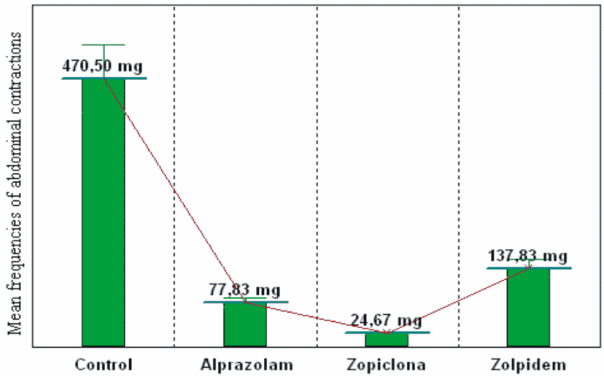

Figure 2 – *The mean frequency of abdominal contractions.*

Table 5 – *Comparison between mean frequencies*

	Control	Alprazolam	Zopiclone	Zolpidem
Control		0.001*	0.001*	0.001*
Alprazolam			0.770	0.699
Zopiclone				0.196

Finally, the mean weight of the cotton wool granuloma was determined three days after inducing the experimental chronic inflammation by the "cotton wool granuloma" method and after administration of these three substances: Alprazolam, Zopiclone and Zolpidem.

In the 4th day the rats were sacrificed and the weight of the cotton pellet was determined in each animal (Table 6 and Figure 3) [9].

Table 6 – *Mean weight of the "cotton wool granuloma" after three days*

Group	Mean weight [mg] ± SD
Control	0.92 ± 0.07
Alprazolam	0.81 ± 0.02
Zopiclone	0.89 ± 0.06
Zolpidem	0.88 ± 0.09

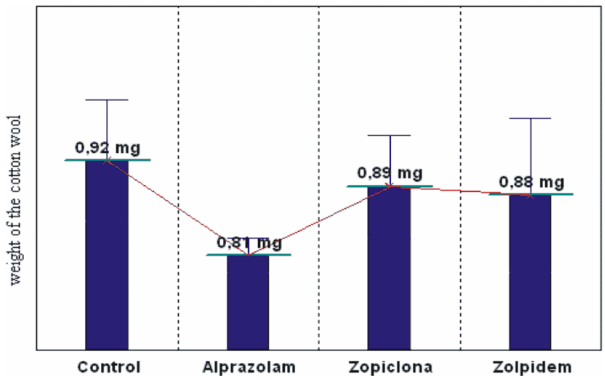

Figure 3 – *The mean weight of the cotton wool granuloma after three days.*

The comparison between the mean weight of the cotton wool granuloma was made between the control group and the groups treated with Alprazolam, Zolpidem and Zopiclone, as well as between the groups treated with these substances (Table 7) [9].

Table 7 – *Comparison between the mean weights of the cotton wool granuloma*

	Control	Alprazolam	Zopiclone	Zolpidem
Control		0.060	0.925	0.843
Alprazolam			0.195	0.274
Zopiclone				0.997

There are no significant differences between the control group and the groups treated with Alprazolam, Zolpidem and Zopiclone, and between the groups treated with drugs. There is a small significant difference (p=0.06) between the Alprazolam group and the control group, meaning that the weight of the cotton wool in the group treated with Alprazolam is less than the weight of the cotton wool in the control group.

Chronic inflammation induced by "cotton wool granuloma" under the administration of Alprazolam, Zolpidem and Zopiclone, was accompanied by a statistically significant decrease in the number of abdominal contractions for 10 minutes, compared with the control group. So, chronic inflammation under the administration of these substances was accompanied by a peripheral analgesic activity in "writhing test". The mechanisms of these effects are not fully elucidated. There are no significant differences between the groups treated with the drugs. After the rats were sacrificed, the histopatologic aspect of granulomatous inflammation was studied as seen in the next figures. The appearance of granulomatous inflammation obtained with the cotton wool is shown in the next figures (Figures 4 and 5).

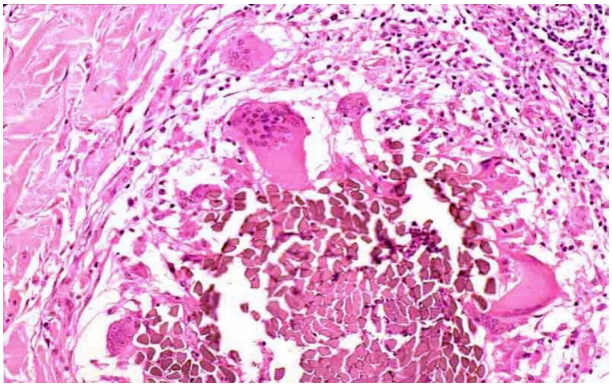

Figure 4 – *Granulomatous inflammation characterized by foreign body giant macrophages (HE stain, ob. ×40).*

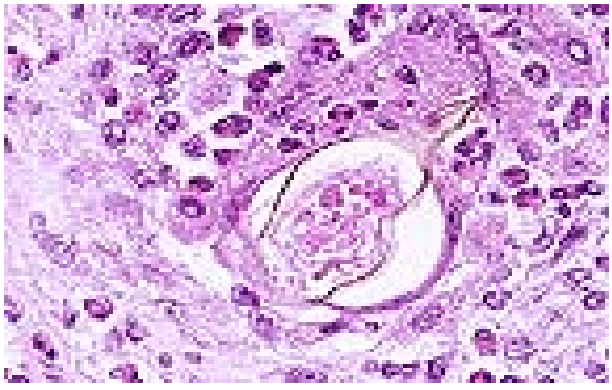

Figure 5 – *Foreign body granuloma (HE stain, ob. ×40).*

Discussion

Chronic inflammation induced by "cotton wool granuloma" under the administration of Alprazolam, Zolpidem and Zopiclone was accompanied by a peripheral analgesic activity in experimental peripheral algesia induced by "writhing test".

The mechanisms of action of Alprazolam, Zolpidem and Zopiclone algesia are still unknown. No doubt, they act as agonists or antagonists on algesia and inflammation mediators (histamine, prostaglandins, cytokines, etc.) [13, 14].

Another explanation is the stimulation of peripheral ω_3-benzodiazepine receptors ("peripheral-type" recaptors) [15–17] that also explains other actions of these substances (the hypoglycemic action in hyperglycemia, the anti-hyperlipidemic action in hyperlipidemia).

These receptors are found in peripheral organs including kidney, heart, several endocrine glands, erythrocytes as well in the CNS.

They are located in the outer mitochondrial membrane and are associated with a voltage-dependent anion channel (VDAC) which is also referred to as "mitochondrial porin".

They have a role in steroidogenesis, immunity, cell growth and differentiation, in cholesterol metabolism, modulation of GABA action in CNS, etc. [9, 16–18].

Conclusions

Chronic inflammation under the administration of Alprazolam, Zolpidem and Zopiclone was accompanied by a peripheral analgesic activity in "writhing test".

There are no significant differences between groups treated with these drugs.

The mechanisms of these effects are not fully elucidated. Some of the theories are: they act as agonists or antagonists on algesia and inflammation mediators and they have a stimulating effect on peripheral ω_3-benzodiazepine receptors ("peripheral-type" receptors).

References

[1] CUPARENCU B, *Farmacologie pentru medici*, vol. I, Ed. Dacia, Cluj-Napoca, 1976.

[2] CUPARENCU B, HORAK J, MARMO E, DE SANTIS D, LAMPA E, LO SASSO C, ROSSI F, *The influence of the peripheral-type benzodiazepine receptor antagonist PK 11195 on blood glucose and serum lipid levels in rats. Interactions with diazepam*, Curr Ther Res, 1991, 49:409–414.

[3] EVERITT BS, *Statistical methods for medical investigations*, 2nd edition, Halsted Press, New York, 1994, 71.

[4] BRUNTON L, LAZO J, PARKER K, *Goodman & Gilman's the pharmacological basis of therapeutics*, 11th edition, McGraw Hill, New York, 2006, 361–393, 581–657.

[5] RADU I, *Metodologie psihologică şi analiza datelor*, Ed. Sincron, Cluj-Napoca, 1993, 91, 180, 263.

[6] STROESCU V, *Bazele farmacologice ale practicii medicale*, ediția a VI-a, Ed. Medicală, Bucureşti, 1999, 931–956.

[7] KATZUNG BG, MASTERS SB, TREVOR AJ, *Basic and clinical pharmacology*, 11th edition, McGraw–Hill, Lange, 2009.

[8] CUPARENCU B, PANTIŞ C, STOICA L, ȚIGLEA MM, *Textbook of fundamental and clinical pharmacology*, Group Promo Publishing House, Oradea, 2004.

[9] ȚIGLEA M, *Effects of some hypnotics and tranquilizers on experimental inflammation*, PhD Thesis, coordinator: Professor Barbu Cuparencu, 2003, 172.

[10] CUPARENCU B, PLEŞCA L, *Actualități în farmacologie şi fiziopatologie*, Ed. Dacia, Cluj-Napoca, 1995, 275–285.

[11] CUPARENCU B, *Efectele medicamentelor cardiovasculare mediate prin sistemul nervos central*, Ed. Medicală, Bucureşti, 1988.

[12] VOGEL HG (ed.), *Drug discovery and evaluation: pharmacological assays*, 2nd edition, Springer-Verlag, Heidelberg, 1997.

[13] LEVINE JD, REICHLING DB, Peripheral mechanisms of inflammatory pain. In: WALL PD, MELZACK R (eds), *Textbook of pain*, Churchill Livingstone, New York, 1999, 59–84.

[14] MUNGIU O, *Tratat de algeziologie*, Ed. Polirom, Iaşi, 2002, 162–188, 253, 263.

[15] ANHOLT RRH, *Mitochondrial benzodiazepine receptors as potential modulators of intermediary metabolism*, Trends Pharmacol Sci, 1986, 7:506–511.

[16] CUPARENCU B, TIMAR M (eds), *The pharmacological receptors*, Ed. Gloria, Cluj-Napoca, 1995.

[17] VERMA A, SNYDER SH, *Peripheral type benzodiazepine receptors*, Annu Rev Pharmacol Toxicol, 1989, 29:307–322.

[18] MOINGEON PH, *Mitochondrial benzodiazepine receptors – biological roles*, IUPHAR 9-th International Congress of Pharmacology, London, 1984, Abstr. 1914.

Corresponding author

Mihaela Zdrîncă, Senior Lecturer, MD, PhD, Department of Pharmacology, Faculty of Medicine and Pharmacy of Oradea, 10 1 Decembrie Square, 410068 Oradea, Romania; Phone +40724–098 041, e-mail: mzdrinca@yahoo.com

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